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Synthesis and pharmacological activities of some new selenium containing nucleoside analogues

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ABSTRACT

Synthesis of selenium-containing nucleoside analogues, derived from some heterocyclic moieties such as pyridineselenol, pyridazineselenol and quinolineselenol derivatives is described herein. These compounds were prepared in a concise and short synthetic route in good yields, by nucleophilic substitution reaction of different selenoheterocyclic compounds with alkyl halides derivatives (e.g. allyl bromide, 3-chloro-propan-1-ol, 3-chloro-3-hydroxy-propan-1-ol and epichlorohydrine as well). Biological screening of the newly synthesized compounds was screened in vitro and in vivo antioxidant studies. The newly synthesized compounds were characterized using the well known spectroscopic tools (IR, UV, ^{1}HNMR, ^{13}CNMR and mass spectroscopy) as well as microanalysis.

Keywords Bioorganic chemistry, Drug research, Heterocycles, Nucleosides, Anti-oxidant, Selenium

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INTRODUCTION

In recent years, considerable attention has been devoted to the synthesis of selenium-containing heterocycles because of their interesting reactivity and potential pharmaceutical applications and therefore, new efficient syntheses are an attractive goal of chemical research [1-10]. Furthermore, in previous work in our laboratory describes the synthesis of pyrimidoselenolo [2,3-b] quinoline,[11] pyrimidoselenolo [2,3-c] pyridazine [12] which indicate that certain compounds possess significant anti-inflammatory and analgesic activities with strong fungicidal effects. Stimulated by our recent work on the synthesis of selenium containing sulfa drugs, [13] and the synthesis of selenium containing amino acid analogues, [14] we decided to expand our interest to the introduction of an organoselenium compounds in the nucleoside analogues framework. From a literature survey indicates that only few publications have mentioned the incorporation of a selenium atom into nucleosides [15]. Consequently, synthesis and biological screening of selenonucleoside analogues may be considered a virgin research area.

MATERIALS AND METHODS

Chemistry

Meting points were determined using a Kofler melting point apparatus and were uncorrected. IR (KBr) spectra were recorded on a Pye-Unicam SP3-100 instrument. 1H NMR spectra were obtained on a Varian EM 390 USA at Assiut University using tetramethylsilane as an internal reference. 13C NMR spectra were recorded on a JNM-LA

spectrometer (400 MHz) at Assiut University. Elemental analyses were obtained on an Elementar Vario EL 1150C analyser. Purity of the compounds was checked by TLC using silica gel plates. Mass spectra were recorded on a JEOL-JMS-AX 500 at Cairo National Research Center and JEOL-JMS 600 at Assiut University, Assiut, Egypt. Elemental analyses were obtained on an Elementer Vario EL 1150C analyzer. Purity of the compounds was checked by TLC.

All physical and spectral data in Table 1.

General Procedures

The selenolo derivatives of pyridine (**1a)**, or pyridazine (**1b)** or quinoline (**1c)** (10 mmol) were heated under reflux with alkyl halides derivatives (e.g. allyl bromide, 3-chloro propan-1-ol, 3-chloro-3-hydroxy-propan-1-ol and epichlorohydrine) (10 mmol) for 2 hrs, in the presence of anhyd. K_2CO_3/ acetone. The reaction mixture was filtrated and the solvent was distilled out completely. The residue was collected and recrystallized from Pet. Ether 40-60^o C.

2-(allylselenyl)-4,6-dimethylpyridine-3-carbonitrile (*2a,* $C_{11}H_{12}N_2Se$**).**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 166.4, 163.1, 154.2, 151.2, 132.7 (CH=CH_2), 124.2 (CH-pyridine), 122.1 (CH=CH_2), 118.7, 33.8 (CH_2CH=CH_2), 21.4 (CH_3), 14.5 (CH_3). MS of compound (2a) exhibited molecular ion peak at MS (70 eV): *m/z* = 253 ([M + 2], 78); 252 ([M + 1] , 58). Calcd. % for $C_{11}H_{12}N_2Se$: C, 52.60; H, 4.82; N, 11.15. Found, %: C, 52.30; H, 4.80; N, 10.89.

3-(allylselenyl)-5,6-diphenylpyridazine-4-carbonitrile (*3a,* $C_{20}H_{15}N_3Se$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 166.4, 160.1, 152.0, 151.2, 146.8, 146.7, 143.10, 140.1, 136.5, 132.7 (CH=CH_2), 129.0, 127.1, 122.1 (CH=CH_2), 118.1, 112.0.7, 33.7 (CH_2CH=CH_2), MS (70 eV): *m/z* = 376 (M^+, 60). Calcd. % for $C_{20}H_{15}N_3Se$: C, 63.83; H, 4.02; N, 11.17. Found, %: C, 63.59; H, 4.00; N, 11.01.

2-(allylselenyl)-4,6-dimethylquinoline-3-carbonitrile (*4a,* $C_{14}H_{15}NSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 204.80, 152.08, 146.82, 146.73, 143.10, 135.13, 131.28 (CH=CH_2) 127.96, 126.41, 121.4, 122.69 (CH=CH_2), 33.20 (CH_2CH=CH_2), 21.53 (CH_3), 18.44 (CH_3). MS (70 eV): *m/z* = 276. ([M^+, 33). Calcd. % for $C_{14}H_{15}NSe$: C, 60.87; H, 5.47; N, 5.07. Found, %: C, 60.75; H, 5.33; N, 4.88.

2-(3-hydroxypropylselenyl)-4,6-dimethylpyridine-3-carbonitrile (*2b,* $C_{11}H_{14}N_2OSe$***)***
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 162.2, 154.2, 151.9, 123.2, 108.3, 115.2, 59.6 (CH_2OH), 34.91(CH_2CH_2OH), 32.6 (CH_3), 24.1 ($CH_2CH_2CH_2OH$), 20.4 (CH_3). MS (70 eV): *m/z* = 270 ([M^+ +1], 55). Calcd. % for $C_{11}H_{14}N_2OSe$: C, 49.08; H, 5.24; N, 10.41. Found, %: C, 48.98; H, 5.11; N, 10.22.

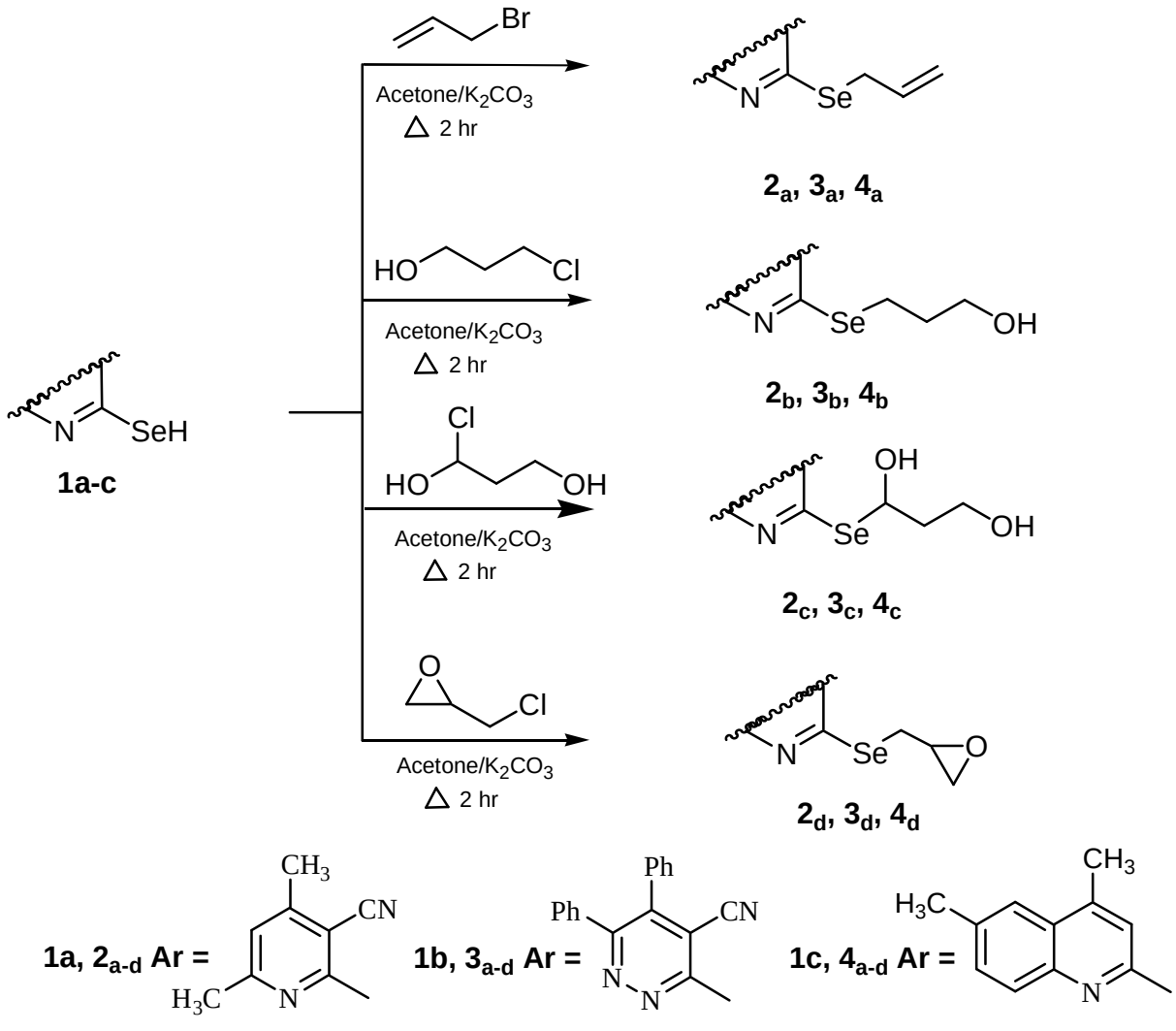
3-(3-hydroxypropylselenyl)-5,6-diphenylpyridazine-4-carbonitrile (*3b,* $C_{20}H_{17}N_3OSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 159.0, 157.2, 141.3, 134.8, 132.4, 130.0, 129.2, 128.6, 127.8, 114.6, 113.6, 59.1 (CH_2OH), 34.9 (CH_2CH_2OH), 32.1 (CH_3), 24.1 ($CH_2CH_2CH_2OH$), 20.4 (CH_3). MS (70 eV): *m/z* = 394 ([M+, 50). Calcd, % for $C_{20}H_{17}N_3OSe$: C, 60.92; H, 4.35; N, 10.66. Found, %: C, 60.69; H, 4.18; N, 10.44.

2-(3-hydroxypropylselenyl)-4,6-dimethylquinoline-3-carbonitrile (*4b,* $C_{14}H_{17}NOSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 159.1, 156.1, 152.2, 143.0, 135.3, 131.4, 128.3, 126.5, 122.9, 59.1 (CH_2OH), 33.3 (CH_2CH_2OH), 28.4 ($CH_2CH_2CH_2OH$), 21.4 (CH_3), 18.4 (CH_3). MS (70 eV): *m/z* = 294 ([M+, 44). Calcd. % for $C_{14}H_{17}NOSe$: C, 57.14; H, 5.78; N, 4.76. Found, %: C, 57.00; H, 5.59; N, 4.49.

2-(1,3-dihydroxypropylselenyl)-4,6-dimethylpyridine-3-carbonitrile (*2c,* $C_{11}H_{14}N_2O_2Se$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 163.1, 159.8, 154.2, 121.2, 113.9, 108.9, 71.6 (Se-CHOH), 63.2 (CH_2OH), 45.4 (-CH_2-CH_2OH), 24.0 (CH_3), 20.2 (CH_3). MS (70 eV): *m/z* = 285 ([M+, 2]. Calcd. % for $C_{11}H_{14}N_2O_2Se$: C, 46.32; H, 4.95; N, 9.82. Found, %: C, 46.14; H, 4.78; N, 9.67.

3-(1,3-dihydroxypropylselenyl)-5,6-diphenylpyridazine-4-carbonitrile (*3c,* $C_{20}H_{17}N_3O_2Se$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 159.2, 154.4, 152.3, 136.5, 130.2, 129.0, 124.5, 128.8, 128.1, 127.0, 118.2, 113.9, 108.0, 71.7 (Se-CHOH), 63.6 (CH_2OH), 45.6 (-CH_2-CH_2OH). MS (70 eV): *m/z* = 410 ([M+, 4]. Calcd. % for $C_{20}H_{17}N_3O_2Se$: C, 58.59; H, 4.18; N, 10.24. Found, %: C, 58.43; H, 4.00; N, 10.01.

Scheme 1: synthesis of compounds 2a-d-4a-d



Scheme 1

2-(1,3-dihydroxypropylselenyl)-4,6-dimethylquinoline-3-carbonitrile (*4c*, $C_{14}H_{17}NO_2Se$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 155.1, 152.0, 143.2, 131.3, 127.8, 126.4, 123.3, 122.7, 112.4, 71.6 (Se-CHOH), 65.1 (CH_2OH), 45.5 (-CH_2-CH_2OH), 21.2 (CH_3), 18.4 (CH_3 MS (70 eV): *m/z* = 310 ([M+, 22]. Calcd. % for $C_{14}H_{17}NO_2Se$: C, 54.20; H, 5.52; N, 4.51. Found, %: C, 54.01; H, 5.46; N, 4.32.

2-(oxiran-2-ylmethylselenyl)-4,6-dimethylpyridine-3-carbonitrile (*2d*, $C_{11}H_{12}N_2OSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 163.2, 154.4, 152.2, 145.9, 126.9, 124.2, 118.2, 115.0, 71.4 (-$CH_2O$$CH_2$), 44.5 ($CH_2OCH_2$), 31.9 (-$CH_2$-$CH_2OHCH_2$). MS (70 eV): *m/z* = 267 ([M^+- 2, 2], 269 (M+, 0.5). Calcd. % for $C_{11}H_{12}N_2OSe$: C, 49.45; H, 4.53; N, 10.48. Found, %: C, 49.28; H, 4.49; N, 10.23.

3-(oxiran-2-ylmethylselenyl)-5,6-diphenylpyridazine-4-carbonitrile (*3d*, $C_{20}H_{15}N_3OSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 157.7, 156.3, 151.2, 146.3, 134.0, 132.3, 130.4, 129.0, 128.6, 128.0, 127.9, 113. 5, 113.0, 70.9 (-$CH_2O$$CH_2$), 45.6 ($CH_2OCH_2$), 29.1 (-$CH_2$-$CH_2$ OH CH_2). MS (70 eV): *m/z* = 392 (M^+, 4]. Calcd. % for $C_{20}H_{15}N_3OSe$: C, 61.23; H, 3.85; N, 10.71. Found, %: C, 61.00; H, 3.55; N, 10.56.

Table 1Physical and spectral data of compounds (2a-d, 3a-d and 4a-d)

Compd. no.	Mp ^{O}C (yield %)	Mol. formula (M/wt)	IR (cm^{-1})	1H NMR (δ, ppm)
2a	40-42 (75)	$C_{11}H_{12}N_2Se$ (251.19)	2200 (CN), 1610 (C=N)	$CDCl_3$: 7.50 (s, 1H, CH-pyridine); 4.31 (s, 1H, CH=CH_2); 4.27 (d, 2H, CH=CH_2); 2.65 (s, 3H, CH_3) 2.50 (s, 3H, CH_3); 2.52 (s, 2H, Se-CH_2).
2b	35-37 (73)	$C_{11}H_{14}N_2OSe$ (269.20)	3400 (OH); 2210 (CN), 1610 (C=N)	$CDCl_3$: 7.50 (s, 1H, CH-pyridine); 4.31 (s, 2H, CH_2 OH); 3.75 (m, 2H, CH_2-CH_2); 3.52 (t, 2H, Se-CH_2); 2.65 (s, 3H, CH_3); 2.50 (s, 3H, CH_3); 2.21 (s, 1H, OH).
2c	40-45(85)	$C_{11}H_{14}N_2O_2Se$ (285.20)	3500 (OH); 2200 (CN);1615 (C=N)	$CDCl_3$: 7.50 (s, 1H, CH-pyridine); 4.41 (s, 2H, CH_2 OH); 4.02 (, 2H, Se-CHOH); 3.75-3.61 (m, 2H, CH_2-CH_2; 2.70 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 2.60(s, 2H, 2OH)
2d	44-46 (77)	$C_{11}H_{12}N_2OSe$ (267.19)		$CDCl_3$: 7.59 (s, 1H, CH-pyridine); 3.28 (s, 3H, CH_3); 3.04 (s, 3H, CH_3); 2.59 (s, 3H, CH-O-CH_2); 2.55 (s, 2H, CH_2).
3a	35-38 (70)	$C_{20}H_{15}N_3Se$ (376.31)	2200 (CN); 1615 (C=N)	$CDCl_3$: 7.31-7.87 (m, 10H, CH-Ar); 5.71 (s, 1H, CH=CH_2); 4.97 (d, 2H, CH=CH_2); 2.50 (s, 2H, Se-CH_2).
3b	33-35 (75)	$C_{20}H_{17}N_3OSe$ (394.33)	2200 (CN); 1620 (C=N)	$CDCl_3$: 7.50-7.22 (m, 10H, CH-Ar); 4.39 (s, 2H, CH_2 OH); 4.05 (m, 2H, CH_2-CH_2); 3.59 (t, 2H, Se-CH_2); 2.51(s, 1H, OH)
3c	32-34 (82)	$C_{20}H_{17}N_3O_2Se$ (410.33)		$CDCl_3$: 7.30-7.80 (m, 10H, CH-Ar); 4.75 (s, 2H, CH_2 OH); 4.52 (s, 2H, Se-CHOH); 3.75-3.85 (m, 2H, CH_2-CH_2); 2.68(s, 2H, 2OH)
3d	40-42 (69)	$C_{20}H_{15}N_3OSe$ (392.31)	3400 (OH); 2210 (CN), 1610 (C=N)	$CDCl_3$: 7.22-7.88 (m, 10H, CH-Ar); 3.09 (s, 3H, CH-O-CH_2); 2.59 (s, 2H, CH_2).
4a	38-40 (73)	$C_{14}H_{15}NSe$ (276.24)	3500 (OH); 2200 (CN)1615 (C=N) 1615 (C=N)	$CDCl_3$: 8.04 (s, 1H, CH-quinoline); 7.31-797(m, 3H, CH-Ar); 4.31 (s, 1H, CH=CH_2); 4.27 (d, 2H, CH=CH_2); 2.70 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 2.50 (s, 2H, Se-CH_2).
4b	44-46 (80)	$C_{14}H_{17}NOSe$ (294.25)		$CDCl_3$: : 8.04 (s, 1H, CH-quinoline); 7.40-7.22 (m, 3H, CH-Ar); 4.31 (s, 2H, CH_2 OH); 3.75 (m, 2H, CH_2-CH_2); 3.52 (t, 2H, Se-CH_2); 2.70 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 2.21(s, 1H, OH)
4c	38-40 (75)	$C_{14}H_{17}NO_2Se$ (310.25)	3500 (OH); 1620 (C=N)	$CDCl_3$: 8.04 (s, 1H, CH-quinoline); 7.40-7.22 (m, 3H, CH-Ar); 4.41 (s, 2H, CH_2 OH); 4.02 (, 2H, Se-CHOH); 3.75-3.61 (m, 2H, CH_2-CH_2); 2.70 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 2.60(s, 2H, 2OH)
4d	35-37 (70)	$C_{14}H_{15}NOSe$ (292.24)	3500 (OH); 2200 (CN) 1615 (C=N)	$CDCl_3$: 8.04 (s, 1H, CH-quinoline); 7.40-7.22 (m, 3H, CH-Ar); 2.70 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 2.59 (s, 3H, CH-O-CH_2); 2.55 (s, 2H, CH_2)

MS of compounds (see experimental Section), 13C NMR of compounds (see experimental section)

Table 2 Effects of selected selenium containing nucleoside on SOD, GSH-S-tranferase , and GSH-Rd in CCl4-intoxicated mice.

Design of treatment	SOD (Units/mg protein)	GST (µmol /mg protein)	GSH-Rd (mg/g protein)
Normal control (Group 1)	$11.65 \pm 0.64^{b,c}$	$2.32 \pm 0.09^{b,c}$	$5.31 \pm 0.11^{b,c}$
CCl_4 + olive oil (Group 2)	$7.15 \pm 0.21^{a,c}$	$1.72 \pm 0.07^{a,c}$	$3.92 \pm 0.20^{a,c}$
Compound 2C (250 mg/kg) + CCl_4 (Group 3)	$9.21 \pm 0.12^{a,b,c}$	$1.90 \pm 0.11^{a,b,c}$	$5.34 \pm 0.32^{a,b,c}$
Compound 2c (500 mg/kg) + CCl_4 (Group 4)	$9.41 \pm 0.73^{a,b,c}$	$1.95 \pm 0.08^{a,b,c}$	$5.45 \pm 0.65^{a,b,c}$
Compound 3b (250 mg/kg) + CCl_4 (Group 5)	$10.32 \pm 0.52^{a,b,c}$	$1.89 \pm 0.15^{a,b,c}$	$5.04 \pm 0.21^{a,b,c}$
Compound 3b (500 mg/kg) + CCl_4 (Group 6)	$10.76 \pm 0.13^{a,b,c}$	$1.91 \pm 0.07^{a,b,c}$	$5.14 \pm 0.25^{a,b,c}$
Compound 3c (250 mg/kg) + CCl_4 (Group 7)	$11.02 \pm 0.14^{a,b,c}$	$1.87 \pm 0.09^{a,b,c}$	$5.34 \pm 0.11^{a,b,c}$
Compound 3c (500 mg/kg) + CCl_4 (Group 8)	$11.21 \pm 0.09^{a,b,c}$	$1.95 \pm 0.15^{a,b,c}$	$5.67 \pm 0.54^{a,b,c}$
Compound 3d (250 mg/kg) + CCl_4 (Group 9)	$10.98 \pm 0.41^{a,b,c}$	$1.91 \pm 0.30^{a,b,c}$	$5.22 \pm 0.12^{a,b,c}$
Compound 3d (500 mg/kg) + CCl_4 (Group 10)	$10.47 \pm 0.19^{a,b,c}$	$1.85 \pm 0.12^{a,b,c}$	$5.32 \pm 0.43^{a,b,c}$
Vitamin E (100 mg/ kg) (Group 11)	$12.15 \pm 0.43^{a,b}$	$2.52 \pm 0.35^{a,b}$	$6.26 \pm 0.53^{a,b}$

Values are mean ± SD, n = 6, a : p < 0.05 compare with vehicle control group, b: p < 0.05 compare with CCl4 group, c: p < 0.05 compare with vitamin E group.

Table 3 Effects of selected selenium containing nucleoside on hepatic GSH-Rd and MDA levels in CCl_4-intoxicated mice.

Design of treatment	GSH-Rd (mg/g protein)	MDA(nmol/mg protein)
Normal control (Group 1)	$15.12 \pm 0.32^{b,c}$	$5.25 \pm 0.13^{b,c}$
CCl_4 + olive oil (Group 2)	$10.19 \pm 0.41^{a,c}$	$8.11 \pm 0.11^{a,c}$
Compound 2C (250 mg/kg) + CCl_4 (Group 3)	$12.85 \pm 0.55^{a,b,c}$	$4.93 \pm 0.45^{a,b,c}$
Compound 2c (500 mg/kg) + CCl_4 (Group 4)	$13.20 \pm 0.24^{a,b,c}$	$3.81 \pm 0.13^{a,b,c}$
Compound 3b (250 mg/kg) + CCl_4 (Group 5)	$11.98 \pm 0.12^{a,b,c}$	$4.14 \pm 0.19^{a,b,c}$
Compound 3b (500 mg/kg) + CCl_4 (Group 6)	$12.71 \pm 0.40^{a,b,c}$	$3.52 \pm 0.23^{a,b,c}$
Compound 3c (250 mg/kg) + CCl_4 (Group 7)	$11.92 \pm 0.24^{a,b,c}$	$4.12 \pm 0.42^{a,b,c}$
Compound 3c (500 mg/kg) + CCl_4 (Group 8)	$11.29 \pm 0.38^{a,b,c}$	$3.16 \pm 0.31^{a,b,c}$
Compound 3d (250 mg/kg) + CCl_4 (Group 9)	$12.34 \pm 0.60^{a,b,c}$	$4.13 \pm 0.42^{a,b,c}$
Compound 3d(500 mg/kg) + CCl_4 (Group 10)	$17.08 \pm 0.51^{a,b,c}$	$3.21 \pm 0.14^{a,b,c}$
Vitamin E (100 mg/ kg) (Group 11)	$12.15 \pm 0.43^{a,b}$	$2.13 \pm 0.20^{a,b}$

Values are mean ± SD, n = 6, [a] : p < 0.05 compare with vehicle control group, [b]: p < 0.05 compare with CCl_4 group, [c]: p < 0.05 compare with vitamin E group.

Fig 1

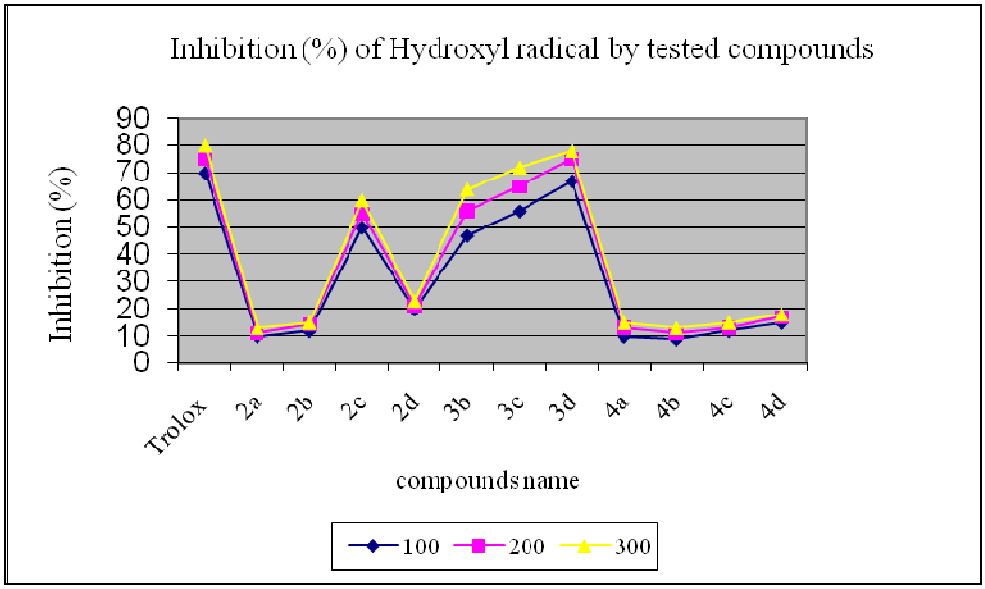
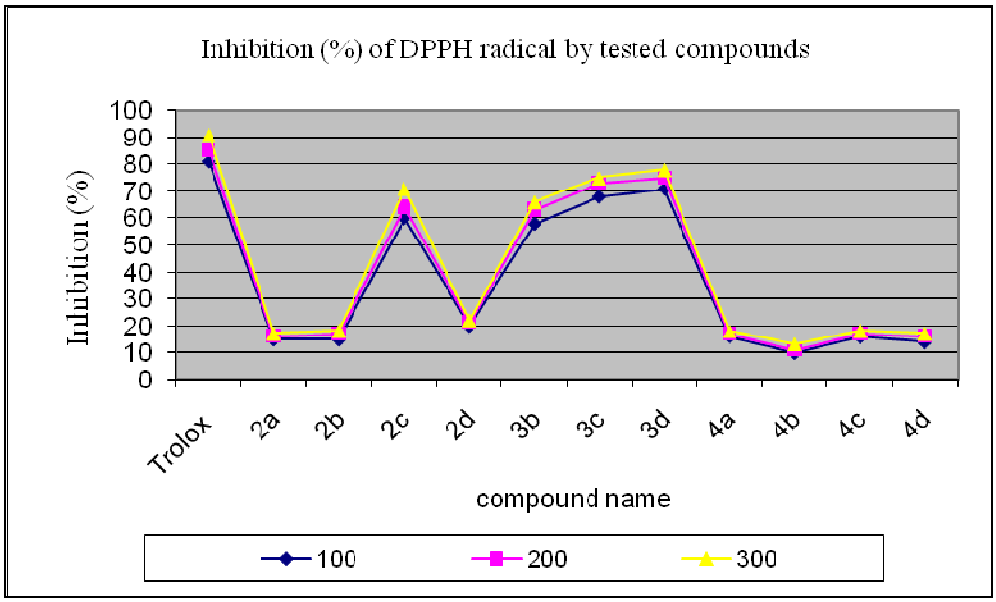


Fig 2



2-(oxiran-2-ylmethylselenyl)-4,6-dimethylquinoline-3-carbonitrile (*4d*, $C_{14}H_{15}NOSe$**)**
13C NMR ($CDCl_3$-d_6, 75 MHz): δ 154.9, 152.2, 145.9, 143.3, 135.1, 126.9, 123.4, 124.2, 118.2, 71.4 (-$CH_2O\underline{CH_2}$), 44.1 ($\underline{CH_2}OCH_2$), 31.8 (-$\underline{CH_2}$-CH_2OHCH_2), 21.6 (CH_3), 18.6 (CH_3). MS (70 eV): *m/z* = 292 ([M^+, 20]. Calcd. % for $C_{14}H_{15}NOSe$: C, 57.54; H, 5.17; N, 4.79. Found, %: C, 57.38; H, 5.01; N, 4.54.

Biological screening
Chemicals
De-oxyribose, thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO,USA). All other chemicals and reagents used were analytical grade.

Animals

Male Albino mice (20 ± 2 g) were obtained from the Department of Animal science, Faculty of Agriculture, Cairo University and animals were quarantined and allowed to acclimate to the laboratory for a week prior to experimentation. The animals were handled under standard laboratory conditions with a 12-h light/dark cycle in a temperature of 25 ± 5 ^{o}C and a relative humidity of 55 ± 5 % controlled room. The basal diet used in these studies was certified feed with appropriate analyses performed by the manufacturer and provided to research Laboratories. Food and water were available ad libitum. Our Institutional Animal Care and Use Committee approved all protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guidelines.

***In vitro* antioxidant potential of synthesized compounds**

DPPH radical scavenging activity

All synthesized compounds were analyzed according to the technique reported [23]. The stock solution of DPPH was prepared by dissolving 24 mg of DPPH with 100 mL in MeOH, and then stored at 20 C in the dark until needed. The working solution was obtained by diluting 10 mL of stock solution with 45 mL MeOH, to obtain an absorbance of 1.1 ± 0.1 units at 515 nm, using a Shimadzu UV-1063 spectrophotometer. Briefly, a volume of 10 µl of different synthesized compounds concentrations (100; 200 and 300 µg/ml), was added to 990 µl of 0.094 mM DPPH in MeOH, to reach 1 mL. Assays were continuously monitored at 515 nm over a 1 h period at 25 ºC. Changes in absorbance were minimal for all samples after 30 min. The antioxidant abilities were expressed as µM Trolox equivalents. Each sample was analyzed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according following equation:

Inhibition % = $[A_B - A_A] / A_B \times 100$

where, A_B is the absorption of the blank sample (t = 0 min) and A_A is the absorption of the tested compounds or standard substance solution (t = 30 min). The EC_{50} value defined as the concentration of antioxidant in the reactive system necessary to decrease the initial DPPH concentration by 50% and was calculated from the results.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to Fenton method described before [24], with slightly modification. Samples with different concentration of synthesized compounds (100; 200 and 300 µg/ml) and ascorbic acid (100;200 and 300 µg/ml) were prepared, then incubated with 9.0 mM $FeSO_4$ (1.0 ml), 0.3% H_2O_2 (1.0 ml) in 0.5 ml salicylic acid–ethanol solution (9.0 mM) for 30 min at 37 °C. Hydroxyl radical was detected by monitoring absorbance at 510 nm. The total volume of the mixture in each tube was made up to 3 mL by adding the required amount of distilled water. Inhibition (I) of deoxyribose degradation in percent was calculated in the following way:

I (%) = 100X (A_0-A_1/A_0); where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test compound.

Acute Toxicity studies.

Toxicity of selected compounds (**2c, 3b, 3c** and **3d**) based on *in vitro* antioxidant potential results was monitored in animal model system by different biochemical profiles including LD_{50} ; GPT and LDH activities. Male Albino mice of 6 animals per group and weighing between 20 and 25 g were administered after overnight fasting with graded doses of (100-1000) mg kg^{-1} b. wt. intra peritoneal of each selected synthesized compounds suspended in DMSO. The toxicological effects were observed after 72 h of treatment in terms of mortality and expressed as LD_{50}. The number of animals dying during the period was noted [25]. Others biochemical parameters determined after 10 days of administration [26] for GPT activity and [27] for LDH activity.

***In vivo* antioxidant activity.**

Antioxidant activity of selected compounds (**2c), (3b), (3c)** and (**3d)** based on *in vitro* antioxidant potential results of eleven synthesized compounds.

Bioassays experimental design.

The protective effect of the selected compounds (**2c), (3b), (3c)** and (**3d)** against Carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in mice was evaluated. The animals were randomly divided into eleven experimental groups of 6 mice each. The first group served as normal control and, during the experiment, received vehicle only

(propylene glycol, 5 ml/kg p. Wt. per day p.o.). Group 2 animals received single dose of equal mixture of carbon tetrachloride and olive oil (50%, v/v, 0.5 ml /kg b, wt. i.p.). on 7 th day, Group 3 to Group 10 animals were pre-treated with selected compounds (**2c), (3b), (3c)** and (**3d)** at 250 and 500 mg/ kg b. Wt. per day p.o., respectively, for 7 days. On 7th day a single dose of carbon tetrachloride (0.5 ml/kg i.p.) was administrated. Group 11 animals were pre-treated with standard drug Vitamin E (100 mg/ kg p. Wt. per day p.o) for 7 days. On 7th day a single dose of equal mixture of carbon tetrachloride and olive oil (50%, v/v, 0.5 ml /kg p. wt. i.p.) was administrated [28].

Twenty-four hours after the last administration, mice were sacrificed. Blood samples were collected and centrifuged at 4000×g at 4°C for 10 min for serums preparation. The liver was removed rapidly, washed and homogenized in ice-cold physiological saline to prepare 10% (w/v) homogenate. Then, the homogenate was centrifuged at 4000×g at 4°C for 10 min to remove cellular debris, and the supernatant was collected for biochemical analysis.

The biochemical assays.
Biochemical parameters were carried out according to the instructions of kits purchased from Diagnostic Company, UK.

Measurement of Glutathione-S-Transferase activity (GST).
GST activity was determined as described [29]. Reaction mixture containing 50 mM phosphate buffer, pH 7.5, 1 mM of 1-chloro- 2, 4-dinitrobenzene (CDNB) and an appropriate volume of compound solution. The reaction was initiated by the addition of reduced glutathione GSH) and formation of S-(2, 4-dinitro phenyl) glutathione (DNP-GS) was monitored as an increase in absorbance at 334 nm. The result was expressed as µmol of CDNB conjugation formed /mg protein /min.

Measurement of Super Oxide Dismutase (SOD) activity.
SOD activity was measured through the inhibition of hydroxylamine oxidation by the superoxide radicals generated in the xanthine–xanthine oxidase system [30].The results were expressed in units/mg protein.

Measurement of Glutathione Reduced (GSH-Rd) levels.
GSH in liver and kidney tissues was determined according to the Ellman method [31], which measures the reduction of 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent) by sulfhydryl groups to 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color. The results were expressed in mg per g protein (mg/g protein).

Measurement of Lipid peroxidation
Lipid peroxidation was measured by the TBARS method based on the formation of malondialdehyde (MDA)examined using the 2-thiobarbituric acid (TBA) as previously method described [32] in liver tissue and expressed as n moles per mg protein using 1,1,3,3-tetraethoxypropane (TEP) as a standard.

Measurement of Protein content
Protein levels were determined spectrophotometrically at 595 nm, using comassie blue G 250 as a protein binding dye [33]. Bovine serum albumin (BSA) was used as a protein standard.

Measurement Statistical analysis
Each of the measurements described was carried out in three replicate experiments and the results are recorded as mean ± standard deviation. The significantly different calculated at level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemistry
We describe herein the synthesis of 2/or 3-substitutedselenyl derived from some heterocyclic moieties such as pyridineselenol, pyridazineselenol and quinolineselenol derivatives (**1a-c)**. For the synthesis of target compounds, first, the heterocyclic selenols (**1a-c)** was prepared as described before [13-16]. After that the derivatives (**2a-d - 4a-d)** (**Scheme 1**) were prepared by nucleophilic substitution reaction of **1a-c** and different alkyl halides such as (allyl bromide, 3-chloro-propan-1-ol, 3-chloro-3-hydroxy-propan-1-ol and epichlorohydrine) in the presence of potassium carbonate in a minimum quantity of acetone as a solvent.

The structures of the newly synthesized compounds (**2a-d - 4a-d)** were confirmed by their spectral data (IR, 1H NMR, ^{13}C NMR and MS) together with elemental analyses. The IR spectra of compounds **2b,c - 3b,c** and **4b,c** showed the most characteristic bands ranging from ν 3400-3500 cm^{-1} due to hydroxyl group. For compounds (**2a,d - 3a,d** and **4a,d)** the data revealed characteristic bands ranging from ν 1610-1615 due to (C=N). The ^{1}H NMR spectra of compounds (**2a), (3a)** and (**4a)** expectedly shows characteristic signals ranging from δ 4.31- 5.71 assignable to ($\underline{CH}$=CH_2); 4.27-4.97 due to (CH=$\underline{CH_2}$) and 2.50-2.52 due to (Se-$\underline{CH_2}$). For compounds (**2b), (3b)** and (**4b)**, the ^{1}H NMR spectra assigned characteristic bands ranging from δ 4.31-4.39 due to ($\underline{CH_2}$OH); 3.75-4.04 assignable to ($\underline{CH_2}CH_2OH$); 3.52-3.59 due to (Se-$\underline{CH_2}$) and 2.21-2.51 due to (OH group). For compounds (**2c), (3c)** and (**4c)** showed signals ranging from δ 4.41-4.75 due to ($\underline{CH_2}$OH); 4.02-4.52 due to (Se-$\underline{CH}$OH); 3.61-3.85 for ($\underline{CH_2}CH_2OH$) and 2.60-2.68 for (2OH group). Finally the ^{1}H NMR spectra of compounds (**2d), (3d)** and (**4d)** showed bands at δ 2.55-2.59 due to ($\underline{CH}O\underline{CH_2}$) and 2.55-2.59 for (Se-$\underline{CH_2}$).

Further confirmation was achieved by the ^{13} C NMR spectra which showed the average signals for compounds (**2a-4a)** at δ 132.7, 122.1 and 33.8 due to ($\underline{CH}$=CH_2), (CH=$\underline{CH_2}$) and ($\underline{CH_2}CH=CH_2$) respectively. For compounds (**2b-4b)** the ^{13} C NMR spectra showed the most important peaks ranging at δ 59.1 due to (CH_2OH), 34.9 due to ($\underline{CH_2}CH_2OH$) and 24.1 for ($\underline{CH_2}CH_2CH_2OH$). For compounds (**2c-4c**) the ^{13} C NMR spectra revealed that δ 71.6, 63.2 and 45.4 for (Se-$\underline{CH}$OH), ($\underline{CH_2}$OH) and (-$\underline{CH_2}$-CH_2OH) respectively. For compounds (**2d-4d)** the ^{13} C NMR spectra assigned signals at δ 70.9, 44.1 and 29.1due to (-$CH_2O\underline{CH_2}$), ($\underline{CH_2}OCH_2$) and (-$\underline{CH_2}$-CH_2OHCH_2) respectively.

Finally, the structures of the synthesized compounds were confirmed by their physical, analytical and spectral data. Results were displayed in Table 1.

Pharmacology

In present study eleven selenium containing nucleoside analogues of pyridine, pyridazine and quinoline derivatives were subject to *in vitro* and *in vivo* antioxidant studies. Averages of antioxidant capacities for synthesized compounds were screened using DPPH and Hydroxyl radical scavenging assays. Toxicity of selected compounds based on *in vitro* antioxidant potential results was monitored in animal model system. Biochemical parameters including LD_{50}, GPT and LDH activities were recorded. *In vivo* antioxidant activity of selected compounds was based on *in vitro* antioxidant potential results of eleven synthesized compounds and toxicity results of the selected compounds. The protective effect against Carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in mice was evaluated. Results indicated that significantly lower activities ($p < 0.05$) of SOD, GSH-S-transferase (GST), and Reduced glutathione (GSH-Rd) were observed in CCl_4-treated group as compared to the normal control group. Also, there were significant increases ($p < 0.05$) in SOD, and GST activities in the treated groups at doses of 250 and 500 mg/kg compared to the CCl_4-treated control group ($p < 0.05$). Lipid peroxidation was measured by the TBARS method based on the formation of malondialdehyde (MDA).Treatment with CCl_4 significantly ($p < 0.05$) decreased the GSH-Rd levels with 33.1 % in the liver as compared to the normal control group. In contrast to the CCl4-treated mice showed significantly increased GSH-Rd levels. The MDA levels in the CCl_4-treated group (8.11 ± 0.11 nmol/mg protein) were significantly ($p< 0.05$) higher than in the normal control group (5.25 ± 0.1).

***In vitro* antioxidant potential of synthesized compounds**

Average of antioxidant capacities for synthesized compounds was screened using two methods; DPPH and Hydroxyl radical scavenging assays. The DPPH free radical assay incorporates a metastable free radical that is capable of accepting hydrogen radicals from antioxidants in solution. The reaction between DPPH and antioxidant can be monitored by the decrease in absorbance of the colored free radical. In the hydroxyl radical assay OH radicals were generated to attack the substrate deoxyribose. The resulting products of the radical attack form a pink chromogen when heated with TBA in acid solution. Since these two methods are based on different mechanisms, they can provide complementary insight into the antioxidant capacity of synthesized compounds in present study.

DPPH radical scavenging assay

Results of inhibition percentage of DPPH radical by eleven tested compounds are plotted in < F**ig. 1** >. Results indicated that compounds 2-(1,3-dihydroxypropylselenyl)-4,6-di- methylpyridine-3-carbonitrile (**2c)** and other three compounds 3-(3-hydroxypropyl- selenyl)-5,6-diphenyl pyridazine-4-carbonitrile (**3b)**, 3-(1,3-dihydroxypropylselenyl)-5,6-diphenyl pyridazine-4-carbonitrile (**3c)** and 3-(oxiran-2-ylmethylselenyl)-5,6-diphenyl-pyridazine-4-carbonitrile (**3d)** have more inhibition effect against DPPH compared to other tested compounds. The EC_{50} values were 83.33; 86.2; 73.53 and 70.4 µg for (**2c), (3b), (3c)** and (**3d)** respectively compared with 61.72 µg

for Trolox. The relative activities compared with Trolox were 73.49, 71.6, 83.56 and 87.14 % for (**2c), (3b), (3c)** and **(3d)** respectively.

Hydroxyl radical scavenging assay

Results of inhibition percentage of hydroxyl radical by eleven tested compounds are plotted in < Fig. 2 >. Results indicated that compounds (**2c), (3b), (3c)** and **(3d)** has more inhibition effect against hydroxyl radical compared to other tested compounds. The EC_{50} values were 100, 106.38, 89.28 and 74.62 µg for (**2c), (3b), (3c)** and **(3d)** respectively compared with 71.72 µg for Trolox. The relative activities compared with Trolox were 71.01, 66.98, 79.77 and 95.94 % for (**2c), (3b), (3c)** and **(3d)** respectively. The average of the antioxidant capacity of synthesized compounds, result indicated that compound (**2c)** and other three compounds (**3b),** (**3c)** and (**3d)** have the highest antioxidant activity.

Acute Toxicity studies.

Toxicity of selected compounds (**2c), (3b), (3c)** and **(3d)** based on *in vitro* antioxidant potential results were monitored in animal model system. Biochemical parameters including LD_{50} ; GPT and LDH activities were recorded by treatment with graded doses of (100-1000) mg kg^{-1} p.wt. of each selected synthesized compounds. Toxicity results indicated that no mortality observed with administration with concentrations range of selected synthesized compounds up to 700 mg kg^{-1} p. wt. Also, result of GPT, and LDH enzyme activities indicated that no-significant effect after administration with selected synthesized compounds up to 700 mg kg^{-1} p. wt. The GPT, an enzyme which allows determining the liver function as indicator on liver cells damage and LDH enzyme is often used as a marker of tissue breakdown [17].

***In vivo* antioxidant activity.**

In vivo antioxidant activity of selected compounds was based on *in vitro* antioxidant potential results of eleven synthesized compounds and toxicity results of the selected compounds (**2c), (3b), (3c)** and **(3d)**. The protective effect against Carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in mice was evaluated for selected compounds. CCl_4-induced hepatic injury has been extensively used in animal models to evaluate the therapeutic potential of drugs and dietary antioxidants. CCl_4 experimentally induced cirrhotic response in animal similar to human cirrhosis of the liver [18-20] respectively. Therefore, The metabolites of CCl_4, trichloromethyl free radicals, are capable of binding to DNA, lipids, proteins or carbohydrates and eventually lead to membrane-lipid peroxidation and finally to cell death [21,22]. Hepatic GSH-Rd and MDA levels, as well as serum activities of SOD, GSH-S-transferase, and GSH-Rd levels were measured to monitor liver injury and as an indicator of antioxidant. The obtained result of activities of SOD, GST, and GSH-Rd levels in CCl_4-intoxicated mice are presented in < Table 1 > SOD and GSH-S-transferase are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals formed in normal condition (food metabolites) or abnormal condition (present of environment pollutants). These enzymes are induced on the generation of free radicals in living cells. Result indicated that significantly lower activities ($p < 0.05$) of SOD, GST, and GSH-Rd were observed in CCl_4-treated group as compared to the normal control group. Also, there were significant increases ($p < 0.05$) in SOD, and GST activities in the treated groups at doses of 250 and 500 mg/kg compared to the CCl_4-treated control group ($p < 0.05$). No significant deference found between used doses (250 and 500 mg/kg) with all tested compounds. The highest increase in SOD and GST, activities rather than GSH-Rd levels was monitored with compound 3-(1,3-dihydroxypropylselenyl)-5,6-diphenylpyridazine-4-carbonitrile (**3c)** as compared to the treated group 2.

Lipid peroxidation and GSH-Rd levels

Lipid peroxidation was measured by the TBARS method based on the formation of malondialdehyde (MDA) which determined using the 2-thiobarbituric acid (TBA). Hepatic GSH-Rd and MDA levels in CCl_4-intoxicated mice are presented in < Table 3 > GSH-Rd acts as a nonenzymatic antioxidant in the detoxification pathway that reduces the reactive toxic metabolites of CCl_4. Treatment with CCl_4 significantly ($p < 0.05$) decreased the GSH-Rd levels with 33.1 % in the liver as compared to the normal control group. In contrast to the CCl_4-treated mice treated with selected selenium containing nucleoside at both doses of 250 and 500 mg/kg showed significantly increased GSH-Rd levels. Also, similar results recorded with vitamin E at dose of 100 mg/kg. The MDA level is widely used as a marker of free-radical mediated lipid peroxidation. The results of the MDA assay are presented in < Table 3 >

MDA levels in the CCl_4-treated group (8.11 ± 0.11 nmol/mg protein) were significantly ($p< 0.05$) higher than in the normal control group (5.25 ± 0.13 nmol/mg protein). Selected selenium compounds at both doses of 250, and 500

mg/kg showed significantly ($p < 0.05$) decreased MDA level with approx. 46 to 51 % in treated mice with the both doses as compared to the normal control group.

CONCLUSION

Present study demonstrates that one of pyridine-moiety 2-(1,3-dihydroxypropylselenyl)-4,6-dimethylpyridine-3-carbonitrile (**2c)** which bearing two hydroxyl groups, and three of pyridazine-moieties 3-(3-hydroxypropyl-selenyl)-5,6-diphenylpyridazine-4-carbonitrile (**3b)**, 3-(1,3-dihydroxypropyl selenyl)-5,6-diphenylpyridazine-4-carbonitrile (**3c)** and 3-(oxiran-2-ylmethylselenyl)-5,6-diphenyl- pyridazine-4-carbonitrile (**3d)** respectively have definite antioxidant effect. The mode of action produce the protective activity against CCl_4 may due to activation of antioxidant enzymes SOD and GST, in addition to the free radicals being released in the liver were effectively scavenged by effective compounds based on the presence of two hydroxyl group (**2c)** and two phenyl ring substitutions (**3b-d)** found either in pyridine or pyridazine rings. This activity in effective compounds is mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

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REFERENCES

[1] G Mugesh, W-W Du Mont, H Sies Chem. Rev., **2001**, 101, 2125-2180.
[2]A V, Gasparian, Y J Yao, J Liu, AY Yemelyanov, L A, Lyakh, J T Slaga, I V Budunova, Mol.Cancer Ther., **2002**, 1, 1079-1090.
[3]J Fleming, A Ghose, P R Harrison, Nut.Cancer, **2001**, 40, 42-49.
[4]A Ghose, J Fleming, K El-Bayoumy, PR, Harrison, Cancer Res., 2**0**01, 61, 7479-7487.
[5] W Wu, K Murakami, M Koketsu, Y Yamada, I Saiki, Anticancer Res.**1999**, 19, 5375-5382.
[6] C Hu, P Zhang, H Li, Z Ji, B Liu, Huaxue Tongbao, 65, **2002**, 162; Chem. Abstr. **2002**, 137, 169 434.
[7] M Koketsu, K Tanaka, Y Takenaka, C D Kwong, H Ishihara, Eur. J. Pharm. Sci. **2002**, 15, 307-310.
[8] Kyoto Encyclopedia of Genes and Genomes, KEGG, Release 40.0, October **2006**.
[9] R Caputa, S Capone, M D Greca, L Longobardo, G Pinto, Tetrahedron Lett, **2007**, 48, 1425-1427.
[10] Narendra Devanaboyina, T.Satyanarayana and B.Ganga Rao, Der pharma Chemical, **2011**, 112-117
[11] Deepa Gor, Pinka Patel, Manan Shah and P. S. Patel, Der pharma Chemical, **2012**, 626-628
[12] Sh H Abdel-Hafez, Eur. J. Med. Chem. **2008**, 43, 1971-1977.
[13] Sh H Abdel-Hafez, M A Hussein, Arch. Pharm. **2008**, 341 (4), 240-246.
[14] Sh H Abdel-Hafez, Russ. J. Bioorg.Chem. **2010**, 36(3), 370–376.
[15] Sh H Abdel-Hafez, H A Saad, M R E Aly, Russ. J. Bioorg.Chem., **2011**, 37 (3) 261–269.
[16] L Antonio Braga, a Wolmar Severo Filho, S Icardo Schwab, E D Oscar Rodrigues, D Lucian, C Hugo Braga, S Diogo Ludtke, Tetrahedron lett. **2009**, 50, 3005-3007.
[17] A A Butt, S Michaels, D Greer, R Clark, P Kissinger, D H Martin, AIDS Read **2002**, 12, 317-321.
[18] Z Taira, K Yabe, Y Hamaguchi, K Hirayama, M Kishimoto, S Ishida, Y Ueda, Food Chem. Toxicol. **2004**, 42, 803–807.
[19] K J, Lee, J H Choi, H G Jeong, Food Chem. Toxicol. **2007**, 45, 2118–2125.
[20] M Rudnicki, M M Silveira, T V Pereira, M R Oliveira, F H Reginatto, F Dal-Pizzol, J C F Moreira, Food Chem. Toxicol. **2007**, 45, 656–66.
[21] L W D Weber, M Boll, A Stampfl, Crit. Rev. Toxicol. **2003**, 33, 105–136.
[22] S Basu, Toxicol. **2003**, 189, 113–127.
[23] W Brand-Williams, M E Cuvelier, C Berset, Lebensmittel-Wissenschaft und-Technol. **1995**, 28, 25–30.
[24] M H Diez, A A Livertoux, Stark, M Wellman-Rousseau, P Leroy, J. Chromat. B: Biomed. Sci. Applications, **2001**, 763 (1–2), 185–193.
[25] M N Ghosh, **1984**. Fundamentals of Experimental Pharmacology. 2nd Edn., Scientific Book Agency, Kolkatta, ISBN: 81-902965-0-7, pp: 175-176.
[26] S Reitman, S Frankel, Am. J. Clin. Pathol. **1957**, 28, 56-56.
[27] H U Bergmeyer, **1974**. Methods of Enzymatic Analysis. 2nd Edn., AcademicPress, New York, ISBN: 0895732424, pp: 534.
[28] S Rai, W Atul, M Kakal, P Bishn, M Pulok, seeds J. of Ethnopharma. **2006**, 104, 322-327.

[29] W H, Habig, M J Pabst, W B Jakoby, J. Biol. Chem. **1974**, 249 (22–25) 7130–7139.
[30] P Kakkar, B Das, P N Visvanathan, Indust. J. Biochem. Biophysics, **1972**, 197,588–590.
[31] G L Ellaman, Arch. Biochem. Biophysics. **1959**, 82, 70–72.
[32] K Yagi, R Rastogi, Anal.Biochem. **1979**, 95, 351-358.
[33] M M Bradford, Anal.Biochem., **1976**, 72, 248 – 254